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TO: Office of Grants and Contracts
National Aeronautics and Space Administration
Washington, D. C.

SUBJ: Semi-Annual Progress Report of NASA Grant No. N G R-04-001-014
for the Reporting Period of 1 Oct 66 - 1 Apr 67

1. During the subject reporting period we have ordered and received the equipment to be used for the research (the last item arrived about one week ago). The lack of equipment purchased with these grant funds, however, has not significantly delayed our progress. We have (in our opinion) accomplished a considerable amount of work during the past six months--a great deal of this using borrowed equipment.

2. Research Accomplished During the Reporting Period.

a. The Effect of Co^{60} Gamma Radiation upon Respiration and ATP Metabolism of L Cells.

Suspensions of L cells were studied in these experiments. The rate of respiration before, during, and after irradiation was measured with an oxygen electrode. The ATP concentrations were measured by the firefly luciferase method. The effects of 60 - 2,500 rads of Co^{60} gamma radiation on the respiratory rate of suspensions of L cells were measured (8 ml of suspension with cell densities of 2×10^5 cells/ml were studied). Doses of 130 rads and above produced a dose-dependent transitory stimulation of the rate of respiration. Doses of 120 rads and below, however, produced a depression of the respiratory rate (no stimulation was observed). When the 120 - 130 "crossover" dose is compared with the single cell survival curve (N = 2.5-3.5, D_0 = 125 - 150 rads), the "crossover" dose lies at the end of the shoulder segment

of the curve. Consequently, those doses which place the colony surviving traction on the log-linear segment were associated with a stimulation of the respiratory rate. Conversely, doses which place the surviving fraction on the shoulder of the curve, produce only a depression of respiration.

Using a similar experimental arrangement, intracellular ATP concentrations were measured during the immediate post-irradiation period. After a dose of 250 rads, there was a transitory rise of the ATP concentration - as compared to non-irradiated controls. When the O_2 uptake data is compared with the ATP results, the O_2 curve leads the ATP curve by about 4 minutes. The entire response period has come and gone within 15 minutes after irradiation.

The following states the hypothesis which we are using to explain the experimental results. The mitochondrial membrane is known to be sensitive to peroxide compounds of several types--especially fatty acid peroxides. Given that peroxide compounds are produced as a consequence of irradiation, perhaps the membrane becomes permeable to some compound which (1) stimulates respiration and (2) is phosphorylated to form a net increase in ATP. The compound ADP seems to be the only substance which can easily fit these criteria. If increased amounts of ADP penetrated the mitochondria, the rate of respiration would be increased (because of the "respiratory control" of ADP). Given an elevated concentration of intramitochondrial ADP, the intramitochondrial ATP concentration would rise because of increased oxidative phosphorylation. After the intramitochondrial ATP concentration reached some maximum level, the egress of ATP from the

mitochondrion into the surrounding cytoplasm would balance the rising intramitochondrial level. Since the rising ATP concentration implies a falling ADP concentration, respiration would decrease with the declining ADP level. As the ATP reached the extramitochondrial cytoplasm, it would enter into reactions which would utilize the ATP (consequently lowering the total intracellular level of ATP) and regenerate the ADP pool. The net result would be a return of the various interacting substances toward normal levels.

We are in process of extending and refining our findings. In cooperation with Dr. Sandberg of the Physiology Department, we are exploring the feasibility of using an analog-to-digital computer processing method to record, to reduce and to process the outputs of the oxygen electrode. Since most of the equipment to perform these experiments is already available, this phase should be accomplished without additional cost to NASA. We are also beginning experiments in which the incorporation of precursors into ATP, DNA, RNA, and protein are measured very soon after irradiation. These data together with the respiration measurements should allow us to learn more about the actual biological significance of the ATP-O_2 changes during the first few post-irradiation minutes.

b. The Effect of Co^{60} Gamma Radiation upon Nucleotide Metabolism of L Cells.

For these experiments we irradiated monolayers of L cells with 2000 rads of Co^{60} radiation. At 24 hours after exposure, $P^{32}O_4$ was added to the medium and a 1 hr. labeling period allowed to elapse before the cells were extracted with perchloric acid (PCA). After extraction the perchlorate ions were removed by neutralization with

KOH. The acid soluble fraction was chromatographed on a Dowex-1 column. These studies showed that NAD, CMP, AMP, GMP, ADP, and ATP were present in measurable quantities.

All fractions were tested for the presence of deoxy sugars by the diphenylamine method; the orcinol reaction was used for identification of ribose. All measurements showed the fractions to contain ribose alone--no deoxy sugars were identified.

The results of the experiments at 24 hours were negative. We are now planning to use these techniques to measure the nucleotide prophiles of cells during the first few post-irradiation minutes. This data together with the macromolecule measurements should increase our understanding of the immediate post-irradiation changes.

c. A Study of a Non-linear Method for Fitting Cell Survival Curves.

The multitarget model for the survival of irradiated cells is given by

$$s = 1-(1- e^{-D/D_o})^n$$

where s is the fraction of cells surviving a dose of D rads. D_o and n are parameters; D_o is that dose required to reduce survival by 0.37 along the log-linear segment while n is the extrapolation number.

Hand graphing methods are usually used to fit curves of this type. Consequently, considerable bias can be injected into the interpretation of the data--depending upon how one tends to "weight" the curve. We are using an iterative technique to fit this model to our data. By applying large sample (asymptotic) theory, we are able to calculate the variances about the estimates of D_o and n. At the present, however, we really do not have a clear understanding of the actual

meaning of these variances.

To study the characteristics of these variances, we are using a digital computer to generate "data" which contains random variability in either the "data" or in the parameters (this is a modified Monte Carlo type of analysis). Given a set of this "data" we then fit curves and calculate the variances--also with the computer. From studies of this type, we hope to be able to determine actually what are the meanings of the variances, to get some notion of their distributions, and to get some insight as to possible weighting factors for the curve fitting.

We plan to extend these studies (together with Dr. Robinette) to seek the answers to such questions as:

(a) What is the optimum number of cells to seed per plate?

(b) What is the optimum number of colonies per plate to get the most information?

(c) How many doses should be studied?

(d) How many plates should be run at each dose?

(e) What responses are necessary to detect changes in the D_o's and n's after the application of radiation modifying drugs.

d. The Effect of Co^{60} Gamma Radiation on ATP Synthesis by L Cells.

For these studies the L cells were suspended in glucose-free Hank's Balanced Salts Solution (HBSS). Immediately after transfer to this deficient medium (no energy source), both the ATP levels and the rate of ATP synthesis (as measured by the incorporation of P^{32}) were rapidly depressed to approximately 80% of the complete medium control. After 100 rads, ATP synthesis is further depressed for a period of some

30 minutes, following the time interval a rapid increase in the rate of ATP synthesis occurs, lasts for 10 to 30 minutes, and then returns to the original depressed level. The ATP levels, however, remain fixed at a level of some 80% of control.

This response has been seen with L cells in three different states of growth (1) log growth, (2) lag phase, and (3) suspension culture.

e. The Effect of 2, 4 Dinitrophenol on ATP Synthesis and Post-Irradiation Survival by L Cells.

The compound 2, 4 dinitrophenol (DNP) uncouples oxidative phosphorylation. Addition of DNP (final concentration 5×10^{-5}M) to suspensions of L cells produces an immediate drop in cellular ATP levels to about 15% of total medium of control. After removal of DNP, the ATP level recovered rapidly to about 60% of control. The incorporation of P^{32} followed the same pattern.

Single cell survival experiments were performed with L cells which had either been treated with DNP or glucose free HBSS. Both of these treatments--which reduce intracellular ATP levels--produced cellular survival greater than cells irradiated in complete medium. The shape of the survival curve of the ATP deficient cells suggest that the shoulder segment is prolonged as compared with controls.

3. Research planned for the next funding period (1 Oct 67 - 30 Sep 68):

Our initial experience strongly suggests that significant biological changes occur during the first post-irradiation hour. With Dr. Robinette's participation we plan to perform a broad group of experiments (which include cell survival, respiration, nucleotide

synthesis, and macromolecule synthesis) during the immediate post-exposure period. The basic cellular responses will be studied under conditions where the cell is rendered more radiosensitive (under increased O_2 tension and after BUdR incubation) and where the cells are made radioresistant (under decreased O_2 tension and in the presence of radioprotectors such as AET).

We plan to continue the broad research plan as outlined in our original protocol. Only now, the emphasis is shifted somewhat to include the more immediate post-irradiation period.

The oxygen electrode data is to be extended by performing paired-dose experiments, and experiments at different dose rates. Also, studies with cells sensitized by BUdR will be performed.

We feel that our first six months of activity have been very productive--we anticipate even more progress in the future.

4. Publications Sponsored by the Grant:

1. J. W. Tyson, J. H. Meade, G. V. Dalrymple, and H. N. Marvin, Some Biomedical Applications of a Non-Linear Curve Fit Method, J. Nuc Med--in press.
2. G. V. Dalrymple, M. L. Baker, and J. L. Sanders, The Effects of CO^{60} Gamma Radiation on Respiration and Survival of L Cells (abst.) Radiat Res--in press; to be presented at the Annual Meeting of the Radiation Research Society, San Juan Puerto Rico, 7 May 67.
3. G. V. Dalrymple, J. L. Sanders, M. L. Baker, and J. L. Schrantz, The Role of Energy Metabolism in the Repair of Radiation Injury by L Cells, J Nuc Med (abst)--in press; to be presented at the annual meeting of The Society for Nuclear Medicine, Seattle, Washington, 21 Jun 67.

4. J. H. Meade and G. V. Dalrymple, A Numerical Method for Estimating the Parameters of Post-Irradiation Cell Survival Curves, J Nuc Med (abst)--in press. To be presented at The Annual Meeting of The Society for Nuclear Medicine, Seattle, Washington, 21 Jun 67.

Presentation

1. "Radiobiological Studies with Cultured Mammalian Cells," G. V. Dalrymple, J. L. Sanders, and J. A. Meade. Presented at Ames Research Center, 24 Feb 67.